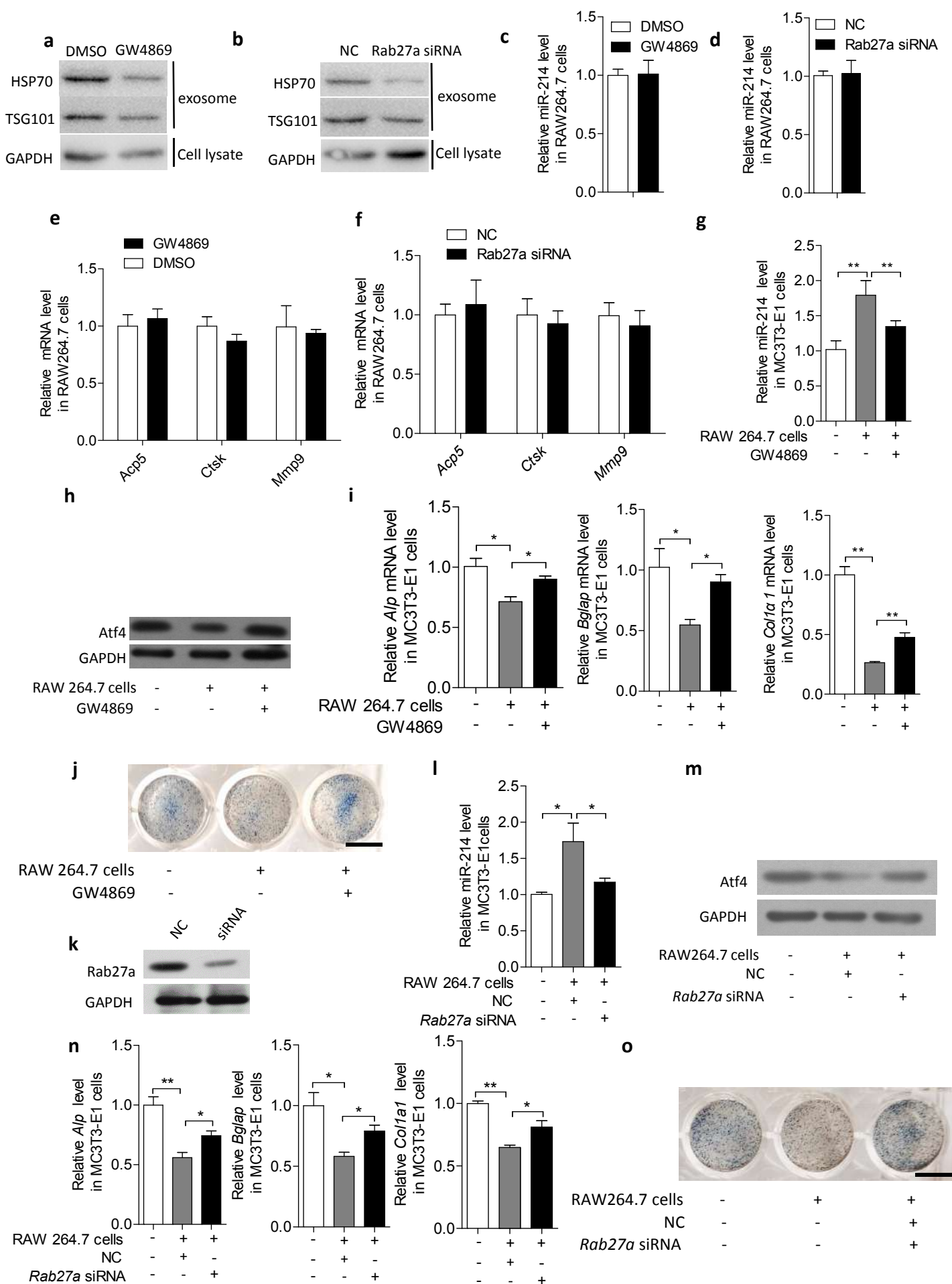


Supplementary Figure 3



Supplementary Figure 3. Inhibition of exosome release from human osteoclast attenuates the inhibitory role of miR-214 on hFOB1.19 cell activity.

(a,b) The levels of HSP70 and TSG101 in exosomes secreted by RANKL-induced RAW264.7 cells treatment with DMSO, GW4869, Rab27a siRNA or NC for 2 days were analyzed by western blot. (c,d) miR-214 levels were analyzed by qRT-PCR in RAW 264.7 cells treatment with GW4869 DMSO, GW4869, Rab27a siRNA or NC. (e,f) *Acp5*, *Mmp9*, *Ctsk* mRNA levels were analyzed by qRT-PCR in RAW264.7 cells treatment with DMSO, GW4869, Rab27a siRNA or NC for 2 days. (g) miR-214 levels were analyzed by qRT-PCR in MC3T3-E1 cells which were co-cultured for 2 days with RANKL-induced RAW 264.7 cells after treatment with the nSMase inhibitor GW4869 (20 μ M). (h) Expression of Atf4 protein in MC3T3-E1 cells which were co-cultured with RANKL-induced RAW 264.7 cells for 2 days with or without GW4869 treatment (20 μ M) for 2 days were analyzed by western blot and were normalized to GAPDH. (i) The mRNA levels of *Alp*, *Bglap* and *Col1 α 1* were analyzed by qRT-PCR in MC3T3-E1 cells which were co-cultured with RANKL-induced RAW 264.7 cells with or without GW4869 treatment. (j) Representative images of Alp staining of osteoblasts after co-cultured with RANKL-induced RAW 264.7 cells with or without GW4869 treatment. (k) Expression of Rab27a protein in RAW 264.7 cells transfected with Rab27a siRNA or NC were analyzed by western blot and normalized to GAPDH. (l) miR-214 level was analyzed by qRT-PCR in MC3T3-E1 cells which were co-cultured with RANKL-induced RAW 264.7 cells transfected with NC and Rab27a siRNA. (m) Expression of Atf4 protein in MC3T3-E1 cells which were co-cultured with RANKL-induced RAW 264.7 cells transfected with NC and Rab27a siRNA were analyzed by western blot and were normalized to GAPDH.

(n) *Alp*, *Bglap* and *Col1 α 1* mRNA level in MC3T3-E1 cells were analyzed by qRT-PCR in MC3T3-E1 cells which were co-cultured with RANKL-induced RAW 264.7 cells transfected with NC and Rab27a siRNA. (o) Representative images of Alp staining of osteoblasts after co-cultured for 6 days with RANKL-induced RAW 264.7 cells transfected with NC and Rab27a siRNA. The culture medium was replaced for fresh medium every 2 days. The data represent the mean \pm SEM of three independent experiments. * $P < 0.05$, ** $P < 0.01$.